Protocol for InterLab Study

NJAU_China

Construction

Use BioBricks to construct devices.
Performe double digestion using SpeI & PstI for promoters, XbaI & PstI for GFP .
The system is as followed:
Buffer 4 ul
ddH_2O 12ul
Enzyme1 2ul
Enzyme2 2ul
DNA 20ul
Transform recombined plasmids into E. coli K-12 DH5-alpha.

Grow Cells for Measurement

Grow following devices and controls:

- Device1: J23101 + I13504 (B0034-E0040-B0015)
- Device2: J23106 + I13504 (B0034-E0040-B0015)
- Device3: J23117 + I13504 (B0034-E0040-B0015)
- Positive Control: BBa_I20270
- Negative Control: 1.E. coli K-12 DH5-alpha without any plasmid added 2.E. coli K-12 DH5-alpha with BBa_R0040 transferred

Followed the protocol provided by iGEM:

Streak out agar plates

LB Agar supplemented with 35ug/ml Chloramphenicol
Streak out 1 plate per device and control
Incubate plates until individual colonies are clearly visible at 37 C

Inoculate liquid culture

Use test tubes (15mm*150mm), oriente at an angle in the incubator, temperature at 37 C, shaking at 200 rpm for 16-18 hours. (Ronghua SHZ-82A Rotary Type Water Bath Thermostated Oscilllator)
3mL Luria Broth supplemented with 35ug/ml Chloramphenicol in each test tube.

Set up biological replicates in triplicate

Plate Reader Measurement

Obtain initial OD600 measurement
TECHCOPM UV2300
Set the spectrophotometer to read OD600, Take the measurement and record it
Dilute samples to an OD600 of 0.5
Calculate the dilution required for each sample, dilute each sample.
Re-measure samples on OD600
If OD600 is within 5% of 0.5, proceed
If OD600 is outside that range, recalculate dilution and remeasure until it's within 5%
Measure samples
TECAN infinite M200 with i-control 1.9
WHB Black Polystyrene Microplates
Set the reader to measure GFP: ex 485, em 528.
Add 100ul to a well for each sample.
Build standard curve by measuring sodium fluorescein with certain concentrations(500, 375, 250, 125, 50, 25, 10, 5, 0 ng/ml).
Measure.